PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CK

(51) International Patent Classification 5 : C07C 9/00, A61K 31/59

A1

(11) International Publication Number: WO 92/05130

(43) International Publication Date: 2 April 1992 (02.04.92)

(21) International Application Number: PCT/US91/06865

(22) International Filing Date: 20 September 1991 (20.09.91)

(30) Priority data:
586,854 21 September 1990 (21.09.90) US

(60) Parent Application or Grant
(63) Related by Continuation
US 586,854 (CIP)
Filed on 21 September 1990 (21.09.90)

(71) Applicant (*for all designated States except US*): LUNAR CORPORATION [US/US]; 313 West Beltline Highway, Madison, WI 53713 (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*) : KNUTSON, Joyce, C. [US/US]; 24 North Prospect Street, Madison, WI 53705 (US). BISHOP, Charles, W. [US/US]; 3641 Okanogan Court, Verona, WI 53573 (US). MORIARTY, Robert, M. [US/US]; 1030 Erie Street, Oak Park, IL 80302 (US).

(74) Agents: GULBRANDSEN, Carl, E. et al.; 25 West Main Street, Suite 300, P.O. Box 2236, Madison, WI 53701-2236 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), BR, CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LU (European patent), NL (European patent), NO, PL, SE (European patent), SU+,US.

Published
With international search report.

(54) Title: NOVEL 1α-HYDROXY VITAMIN D_4 AND NOVEL INTERMEDIATES AND ANALOGUES

(57) Abstract

Novel 1α-hydroxy vitamin D_4 and novel analogues, 1,25 dihydroxy vitamin D_4 and 1,24 dihydroxy vitamin D_4 which are useful as active compounds of pharmaceutical compositions for the treatment of disorders of calcium metabolism. Preparation of the novel 1α-hydroxy vitamin D_4 starts from ergosterol which is converted in six steps to 22,23-dihydroergosterol. 22,23-dihydroergosterol was irradiated to yield vitamin D_4 which is converted in four steps to 1α-hydroxy vitamin D_4 using a cyclovitamin procedure which produces the novel intermediates, vitamin D_4 tosylate, 3,5 cyclovitamin D_4 and 1α-hydroxy cyclovitamin D_4. 1,25 dihydroxy vitamin D_4 and 1,24 dihydroxy vitamin D_4 are isolated as biological products of the metabolism of novel 1α-hydroxy vitamin D_4 using cultured human liver cells.

+ See back of page

+ DESIGNATIONS OF "SU"

Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MN	Mongolia
BE	Belgium	GA	Gabon	MR	Mauritania
BF	Burkina Faso	GB	United Kingdom	MW	Malawi
BG	Bulgaria	GN	Guinea	NL	Netherlands
BJ	Benin	GR	Greece	NO	Norway
BR	Brazil	HU	Hungary	PL	Poland
CA	Canada	IT	Italy	RO	Romania
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland	KR	Republic of Korea	SN	Senegal
CI	Côte d'Ivoire	LI	Liechtenstein	SU+	Soviet Union
CM	Cameroon	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
DE	Germany	MC	Monaco	US	United States of America
DK	Denmark				

NOVEL 1α-HYDROXY VITAMIN D_4
AND NOVEL INTERMEDIATES AND ANALOGUES

TECHNICAL FIELD

This invention relates to biologically active vitamin D_4 compounds. More specifically, this invention relates to novel 1α-hydroxy vitamin D_4 and novel intermediates used in its synthesis, novel 1,25 dihydroxy vitamin D_4, and novel 1,24 dihydroxy vitamin D_4.

This invention also relates to a pharmaceutical composition which includes a pharmaceutically effective amount of the novel 1α-hydroxy vitamin D_4 compounds, and to a method of controlling abnormal calcium metabolism by administering a pharmaceutically effective amount of the novel compounds.

BACKGROUND

Vitamin D is known to be important in the regulation of calcium metabolism in animals and man. See, Harrison's Principals of Internal Medicine: Part Eleven, "Disorders of Bone and Mineral Metabolism, Chapter 335," E. Braunwald, et al., (eds.), McGraw-Hill, New York, 1987, pp. 1860-1865. The two most commonly known, useful forms of vitamin D are vitamin D_3 and vitamin D_2. Vitamin D_3 is synthesized endogenously in the skin of animals and man, whereas vitamin D_2 is the form of vitamin D supplied by plants. Vitamin D_2 differs from vitamin D_3 in that it contains a double bond between C22 and C23 and further contains a C24-methyl group. In man and rats, vitamin D_3 and vitamin D_2 have equivalent biopotency.

Vitamin D_4, also known as irradiated 22,23-dihydro-ergosterol or 22,23-dihydro vitamin D_2 or 22,23-dihydroergocalciferol, differs from vitamin D_3 in that it contains a C24 methyl group. Vitamin D_4 was first described in 1936. See, Grab, W., Z.Physiol. Chem., 243:63 (1936); McDonald, F.G., J. Biol. Chem., 114:IVX (1936). See also, Windaus, A. and Trautmann, G., Z. Physiol. Chem., 247:185-188 (1937). These references report some disagreement as to the level of biological activity of the vitamin suggesting that in the rat, vitamin D_4 is one-third or three-fourths as active as vitamin D_3

and in the chick, either one-tenth or one-fifth as active as vitamin D_3.

A more definitive study of the biological activity of vitamin D_4 was made by DeLuca, et al., in 1968. DeLuca, et al., Arch. Biochem. Biophys., 124:122-128 (1968). There, the authors confirmed that vitamin D_4 was less active than vitamin D_3. DeLuca, et al., report that, in their hands, vitamin D_4 is two-thirds as active as vitamin D_3 or vitamin D_2 in the rat, and one-fifth as active as vitamin D_3 in the chick.

DeLuca, et al., make reference to the fact that "[t]he synthesis of vitamin D_4 has apparently been little used since it was first described by Windhaus and Trautmann," and comment, "[t]his is perhaps due to the fact that vitamin D_4 is only of academic interest."

To applicants' knowledge, vitamin D_4 has remained "only of academic interest" as applicants are unaware of any further study of vitamin D_4 since that reported by DeLuca, et. al. In fact, The Merck Index states with respect to vitamin D_4, "Its biological activity seems doubtful." Merck Index, S. Budavari (ed.), 11th ed., Merck & Co., Rahway, N.J., (1989) pp. 1579, #9930.

Since DeLuca, et. al., discovered the active form of vitamin D_3, 1,25-dihydroxy vitamin D_3, (U.S. Patent No. 3,697,559) and its synthetic precursor, 1α-hydroxy vitamin D_3, (U.S. Patent 3,741,996), most interest has centered on developing therapeutic uses of these active vitamin D_3 metabolites. Unfortunately, while the vitamin D_3 metabolites held great promise as therapeutic agents, this promise has never been fully realized because of the extreme toxicity of these agents. For example, toxicity limits the efficacy of vitamin D_3, its active forms and analogs, to prevent bone loss or restore lost bone. Many studies indicate that at dosages required for these agents to be effective in bone loss prevention or restoration, hypercalcemia and hypercalciuria are problems. It has been reported that 1α-hydroxy vitamin D_3 at a daily dose of 2 μg/day (which has been shown in some studies to be effective in preventing loss of bone) causes toxicity in approximately 67% of patients. What is needed is a biopotent vitamin D metabolite of low toxicity, such that the drug is practical as a

therapeutic agent.

SUMMARY OF THE INVENTION

The novel compounds of the invention, 1α-hydroxy vitamin D_4, 1,25-dihydroxy vitamin D_4 and 1,24-dihydroxy vitamin D_4, are bioactive forms of vitamin D_4. The present inventors have discovered that these active forms of vitamin D_4 display much greater biopotency than would be predicted on the basis of the previously reported bioassays of vitamin D_4. The present inventors have also discovered, that the bioactive novel compounds are less toxic than would be predicted on the basis of their biopotency. This combination of high activity with low toxicity makes the compounds of the invention useful as therapeutic agents in the treatment of disorders of calcium metabolism. The novel compounds of the invention are advantageously used as the active compounds of pharmaceutical compositions for diseases induced by abnormal metabolism of calcium.

In order to study the novel compounds of the invention, it was necessary to develop processes for their production. One alpha-hydroxy vitamin D_4 was made synthetically and in the course of that synthesis, novel intermediates were also produced. 1,25-dihydroxy vitamin D_4 and 1,24-dihydroxy vitamin D_4 are isolated as biological products of the metabolism of 1α-hydroxy vitamin D_4.

Other advantages and a fuller appreciation of the specific adaptations, compositional variations, and physical and chemical attributes of the present invention will be gained upon an examination of the following detailed description of the invention, taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will hereinafter be described in conjunction with the appended drawings, wherein like designations refer to like elements throughout and in which:

Figure 1 illustrates preparative steps for the synthesis of vitamin D_4; and

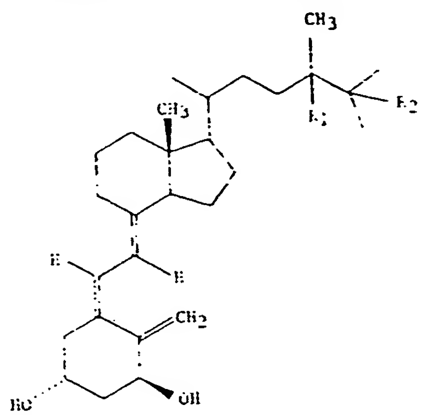
Figure 2 illustrates preparative steps for the synthesis of 1α-hydroxy vitamin D_4 starting with vitamin D_4.

DETAILED DESCRIPTION

The present invention provides synthetic 1α-hydroxy vitamin D_4 (1α-OH-D_4) compounds as well as tosylated and cyclic derivatives of vitamin D_4.

As used herein, the terms "biological activity" or "biologically active" are meant to refer to biochemical properties of compounds such as affecting metabolism, e.g., affecting serum calcium concentration, or binding to an appropriate receptor protein, e.g., binding to vitamin D recepter protein.

In one of its aspects, the invention encompasses biologically active compounds of the general formula (I):



(I)

wherein R_1 is either H or OH, and R_2 is either H or OH, and salts, hydrates and solvates thereof. Preferred compounds among those of formula (I) are those in which R_1 and R_2 are both H; R_1 = OH and R_2 = H; and R_1 = H and R_2 = OH.

In another aspect, the invention involves the preparation of compounds of formula (I). Synthesis of 1α-hydroxy vitamin D_4, i.e., compounds of formula (I) wherein R_1 and R_2 are H, is accomplished according to the schema presented in Figures 1 and 2. As seen in Figure 1, the synthesis uses ergosterol as the starting material. Ergosterol undergoes side chain saturation in a six-step process to yield 22,23-dihydroergosterol (VIII) using a procedure similar to that of Barton, et al., JCS Perkin I, 1976, 821-826. The 22,23-dihydroergosterol is then irradiated as described in Windaus, et al., Z. Physiol. Chem., 1937, 147:185, to yield vitamin D_4 [22,23-dihydroergocalciferol] (IX). As seen in Figure 2, vitamin D_4 is then hydroxylated in a

four-step process to yield 1α-hydroxy vitamin D_4 using a procedure similar to that described by Paaren, et al., J. Org. Chem., 1980, 45:3253.

Specifically, ergosterol is acetylated to form the 3β-acetate. This ergosterol acetate is subjected to hydroxyhalogenation at the 5,6 double bond to form the 6α-chloro-5α-hydroxy derivative. This chlorohydrin is reduced and reacetylated to the 5α-hydroxy (i.e., 5α-ol) derivative. The 5α-ol is subjected to hydrogenation to saturate the side chain. The resulting 3β-acetoxyergost-7en-5α-ol is reduced to 22,23 dehydroergosterol acetate which is in turn reduced to yield 22,23 dehydroergosterol. The 22,23 dehydroergosterol is then irradiated to form vitamin D_4. Vitamin D_4 is then tosylated to yield 3β-tosyl vitamin D_4. The tosylate is displaced by solvolysis to yield the 6-methoxy-3,5-cyclovitamin D_4. The cyclovitamin D_4 is subjected to allylic oxidation to form the 1α-hydroxy cyclovitamin derivative. The 1α-hydroxy cyclovitamin derivative is sequentially solvolyzed and subjected to a Diels-Alder-type reaction which removes the 5-methoxy group and separates the 1α-hydroxy vitamin D_4 (5,6-cis) from the 5,6 trans-1α-hydroxy vitamin D_4.

The 1,24 dihydroxy vitamin D_4 and 1,25 dihydroxy vitamin D_4 metabolites of 1α-hydroxy vitamin D_4, are synthesized by incubating the 1α-hydroxy derivatives with human liver cells, culturing the cells, and recovering the 1,24 dihydroxy or 1,25 dihydroxy vitamin D_4. Using vitamin D receptor protein binding tests, these metabolites are determined to be biologically active.

The compounds of formula (I) have been found to possess valuable pharmacological activity, namely, as controlling agents for calcium metabolism, especially serum calcium concentrations. Specifically, the compounds of formula (I) increase serum calcium concentrations in rats with vitamin D deficiency. It has also been found that the compounds of formula (I) have low toxicity, which enhances their pharmaceutical properties. Compounds of formula (I) have a toxicity, as measured by the LD_{50} test, which is similar to that of corresponding vitamin D_2 compounds and lower than that of corresponding vitamin D_3 compounds. Thus, the compounds of the invention are applicable

to various clinical and veterinary fields, and are particularly useful for the treatment of abnormal metabolism of calcium and phosphorus.

In a further aspect, the invention entails a method of controlling calcium metabolism, such as for treating abnormal calcium metabolism caused, e.g., by liver failure, renal failure, gastrointestinal failure, etc. The compounds of formula (I) can be used to treat prophylactically or therapeutically vitamin D deficiency diseases and related diseases, for example, renal osteodystrophy, steatorrhea, anticonvulsant osteomalacia, hypophosphatemic vitamin D-resistant rickets, osteoporosis, including postmenopausal osteoporosis, senile osteoporosis, steriod-induced osteoporosis, and other disease states characteristic of loss of bone mass, pseudodeficiency (vitamin D-dependent) rickets, nutritional and malabsorptive rickets, osteomalacia and osteopenias secondary to hypoparathyroidism, post-surgical hypoparathyroidism, idiopathic hypothyroidism, pseudoparathyroidism, and alcoholism. The compounds of formula (I), preferably those wherein R1 or R2 is OH, such as 1α,24 dihydroxy vitamin D_4, are of value for the treatment of hyperproliferative skin disorders such as psoriasis.

The compounds of formula (I) are useful as active compounds in pharmaceutical compositions having reduced side effects and low toxicity as compared with the known analogs of active forms of vitamin D_3, when applied, for example, to diseases induced by abnormal metabolism of calcium. These pharamaceutical compositions constitute another aspect of the invention.

The pharmacologically active compounds of this invention can be processed in accordance with conventional methods of pharmacy to produce medicinal agents for administration to patients, e.g., mammals including humans. For example, the compounds of formula (I) can be employed in admixtures with conventional excipients, e.g., pharmaceutically acceptable carrier substances suitable for enteral (e.g., oral), parenteral, or topical application which do not deleteriously react with the active compounds.

Suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions, alcohols, gum arabic,

vegetable oils (e.g., corn oil, cottonseed oil, peanut oil, olive oil, coconut oil), fish liver oils, oily esters such as Polysorbate 80, polyethylene glycols, gelatine, carbohydrates (e.g., lactose, amylose or starch), magnesium stearate, talc, silicic acid, viscous paraffin, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, hydroxy methylcellulose, polyvinyl pyrrolidone, etc.

The pharmaceutical preparations can be sterilized and, if desired, be mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or one or more other active compounds, for example, vitamin D_3 or D2 and their 1α-hydroxylated metabolites, conjugated estrogens or their equivalents, anti-estrogens, calcitonin, biphosphonates, calcium supplements, cobalomin, pertussis toxin and boron.

For parenteral application, particularly suitable are injectable, sterile solutions, preferably oily or aqueous solution, as well as suspensions, emulsions, or implants, including suppositories. Ampoules are convenient unit dosages.

For enteral application, particularly suitable are tablets, dragees, liquids, drops, suppositories, lozenges, powders, or capsules. A syrup, elixir, or the like can be used if a sweetened vehicle is desired.

Sustained or directed release compositions can also be formulated, e.g., liposomes or those in which the active compound is protected with differentially degradable coatings, e.g., by microencapsulation, multiple coatings, etc.

For topical application, suitable nonsprayable viscous, semi-solid or solid forms can be employed which include a carrier compatible with topical application and having a dynamic viscosity preferably greater than water. Suitable formulations include, but are not limited to, solutions, suspensions, emulsions, creams, ointments, powders, liniments, salves, aerosols, transdermal patches, etc., which are, if desired, sterilized or mixed with auxiliary agents, e.g., preservatives, stabilizers, demulsifiers, wetting agents, etc.

For rectal administration, compounds are formed into a pharmaceutical composition containing a suppository base such as

cacao oil or other triglycerides. To prolong storage life, the composition advantageously includes an antioxidant such ascorbic acid, butylated hydroxyanisole or hydroquinone.

Oral administration of the pharmaceutical compositions of the present invention is preferred. Generally, the compounds of this invention are dispensed by unit dosage form comprising about 0.5 μg to about 25 μg in a pharmaceutically acceptable carrier per unit dosage. The dosage of the compounds according to this invention generally is about 0.01 to about 0.5 μg/kg/day, preferably about 0.04 to about 0.3 μg/kg/day.

It will be appreciated that the actual preferred amounts of active compound in a specific case will vary according to the efficacy of the specific compound employed, the particular compositions formulated, the mode of application, and the particular situs and organism being treated. For example, the specific dose for a particular patient depends on the age, body weight, general state of health, sex, on the diet, on the timing and mode of administration, on the rate of excretion, and on medicaments used in combination and the severity of the particular disorder to which the therapy is applied. Dosages for a given host can be determined using conventional considerations, e.g., by customary comparison of the differential activities of the subject compounds and of a known agent, such as by means of an appropriate conventional pharmacological protocol.

In a still further aspect, the compounds of the present invention can also be advantageously used in veterinary compositions, for example, feed compositions for domestic animals to treat or prevent hypocalcemia. Generally, the compounds of the present invention are dispensed in animal feed such that normal consumption of such feed provides the animal about 0.01 to about 0.5 μg/kg/day.

The following examples are to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. In the following examples, all temperatures are set forth in degrees Celsius; unless otherwise indicated, all parts and percentages are by weight. Proton nuclear magnetic (^1H NMR) spectra were recorded with an IBM Sy-200(200 mHz) and a Bruker Am--400(400 mHz) with aspect

3000 Computer in $CDCl_3$ solutions with $CHCl_3$ as an internal standard. Infrared spectra were recorded with a Fourier transform (FTIR) using samples as potassium bromide (KBr) pellets or as liquids. Mass spectra were recorded with a Finnigan MAT-90 mass spectrometer at 20 eV/CI. Melting points are determined on a Hoover-Thomas (capillary) Uni-Melt and a Fisher-Johns melting point apparatus (cover-slip type).

Example 1: Synthesis of 1α-hydroxy vitamin D_4

Ergosterol (II) was converted to ergosterol acetate (III) by dissolving 100 g (0.25 mol) ergosterol in 600 ml of anhydrous pyridine and 68 ml (0.7 mol) acetic anhydride. The solution was stirred overnight at room temperature after which time the solution was cooled by adding 1.2 L ice, causing a precipitate to form. The precipitate was washed five times with 400 ml portions of water, then once with 400 ml of CH_3CN. The resulting product was air dried to yield 79 g (71%) of ergosterol acetate as a white crystalline solid and had the following characteristics: melting point (m.p.): 169-171°C; ^1H NMR: (400 MHz, $CDCl_3$), δppm 2.05 (3H, s, 3β-CH_3CO), 4.65-4.75 (1H, m, 3α-H) 5.15-5.25 (2H, m, 22-H and 23-H), 5.4 (1H, d, 6-H), 5.6 (1H, d, 7-H); FTIR [KBr]: 1734 cm^{-1} (C=O stretching) 968 cm^{-1} (C-H bending).

Ergosterol acetate (III) (26 gm, 0.062 M) was dissolved in 2.5 L of freshly distilled deoxygenated toluene. To this solution 9 ml (0.111 mol) chromyl chloride dissolved in 240 ml dry CH_2Cl_2 was added under nitrogen at -78°C over a thirty minute period. The reaction system was stirred at -78°C for an additional fifteen minutes, and then 62 ml of a saturated solution of sodium borohydride in ethanol was added in one portion. After stirring at -78°C for an additional fifteen minutes, the reaction solution was poured into a two phase system of 3N hydrochloric acid (3L) and benzene (3L). The organic layer was separated, then washed with water (2L), twice with a brine solution (2 x 1L) and then dried with anhydrous $MgSO_4$. The dried solution was filtered and concentrated in vacuo. The crude crystalline product was then treated with CH_3CN (280ml) and filtration of the thus formed slurry yielded 12.5 g (41%) of white crystalline 3β-Acetoxy-6α-chloroergosta-7,22-dien-5α-ol

(IV) and had the following characteristics: m.p.: 190-192°C; ^1H NMR: (400 MHz, $CDCl_3$), δppm 2.05 (3H, s, 3β-OAc), 4.65 (1H, d, 6β-H), 5.1 (1H, s, 7-H), 5.1-5.3 (2H, m, 22-H and 23-H); FTIR [KBr]: 1732 cm^{-1} (C=O stretching), 968 cm^{-1} (C-H bending), 3437 cm^{-1} (O-H stretching).

The 3β-Acetoxy 6α-chloroergosta-7,22-dien-5α-ol (IV) (21.4 g, 0.044 mol) in dry THF (900 ml) was added slowly to a stirred suspension of lithium aluminium hydride (2.66 g, 0.07 mol) in dry THF (750 ml) at room temperature under nitrogen. The mixture was refluxed for three hours and cooled to 0°C. Excess hydride was decomposed with saturated Na_2SO_4 solution. Filtration through anhydrous Na_2SO_4 and evaporation of the filtrate gave a solid, which was treated directly with acetic anhydride (110 ml) and dry pyridine (220 ml) at 0°C. Removal of solvent under reduced pressure yielded the acetate (12.75 g, 61%), 3β-Acetoxyergosta-7,22-dien-5α-ol (V) and had the following characteristics: m.p.: 229-232°C; FTIR [KBr] 1736 cm^{-1} (C=O stretching), 3460 cm^{-1} (O-H stretching), 972 cm^{-1} (C-H bending).

3β-Acetoxyergosta-7,22-dien-5α-ol (V) (2.5 g, 0.0055 mol) was shaken for sixteen hours with freshly prepared PtO_2 (0.5 g) in ethyl acetate (820 ml) under H_2 gas (15 psi). The catalyst was removed by filtration and evaporation of the filtrate gave the crude acetate which was dissolved in CH_2Cl_2 and chromatographed on silica gel. Elution with CH_2Cl_2 gave substantially pure 3β-Acetoxyergost-7-en-5α-ol (VI) (2.15 g, 85%) as a white crystalline material and had the following characteristics: m.p.: 228-232°C; ^1H NMR: (400 MHz, $CDCl_3$), δppm 2.05 (3H, s, 3β-OAc), 5.05-5.20 (2H, m, 3α-H and 7-H); FTIR [KBr]: 1736 cm^{-1} (C=O stretching), 3462 cm^{-1} (O-H stretching).

Redistilled thionyl chloride (9.7 ml) in dry pyridine (170 ml) was added to compound 3β-Acetoxyergost-7-en-5α-ol (VI) (12.0 g, 0.0262 mol) in dry pyridine (800 ml) at 0°C under nitrogen. After 2.5 hours, the solution was diluted with ice cold H_2O (1.5 L) and extracted with two portions of ether (2.5 L + 1.5 L). The combined ether extracts were washed with a $NaHCO_3$ solution (1.0 L x 2), then 1N HCl (1.5 L x 2) and then water (1 L). The ether solution was dried with $MgSO_4$, and after filtration, evaporated under reduced pressure to yield a crude product which

was converted to a slurry with CH_3CN (100 ml). The product was collected by filtration and recrystallized from CH_3CN to yield 4.5 g. (39%) of a white crystalline 22,23-dihydroergosteryl acetate (VII) and had the following characteristics: m.p.: 144-147°C; ^{1}H NMR: (400 MHz, $CDCl_3$), δppm 2.05 (3H, s, 3β-OAc), 4.65-4.75 (1H, m, 3α-H), 5.4 (1H, d, 6-H), 5.6 (1H, d, 7-H); FTIR [KBr]: 1734 cm^{-1} (C=O stretching).

22,23-dihydroergosteryl acetate (VII) (4.8 g, 0.011 mol) was added at once to a stirred suspension of lithium aluminium hydride (2.5 g, 0.066 mol) in dry ether (1.1 L) at room temperature. The mixture was stirred for two hours at room temperature. 5N NaOH was added to destroy excess lithium aluminium hydride and H_2O (500 ml) was then added. The aqueous solution was then extracted with four 250 ml portions of ether. The combined ether extracts and combined organic layer were washed with brine solution (1 L), then dried with Na_2SO_4. Evaporation of ether under reduced pressure gave the compound, 22,23-dihydroergosterol, (VIII) (4.1 g, 94%) as a white crystalline material and had the following characteristics: m.p.: 147-150°C; ^{1}H NMR: (400 MHz, $CDCl_3$), δppm 3.6-3.7 (1H, m, 3α-H), 5.4 (1H, d, 6H), 5.6 (1H, d, 7-H); FTIR [KBr]: 3400 cm^{-1} (O-H stretching).

22,23-dihydroergosterol (VIII) (2.0 g, 5.0 mmol) was dissolved in a solution of diethyl ether and benzene (4:1, 600 ml) and irradiated (Hannovia immersion lamp, 450 watts) with stirring under argon in a water-cooled quartz vessel for three hours. The solution was concentrated in vacuo to yield a gummy solid, which was redissolved in 100 ml. of ethanol and heated at reflux under argon for eight hours. Then, the solution was concentrated in vacuo and the residue was adsorbed on a silica gel column and eluted with 30% ethyl acetate in hexane to afford vitamin D_4 (22,23-dihydroergocalciferol) (IX) with a yield of 1.2 g. (60%) and with the following characteristics: ^{1}H NMR: (400 MHz, $CDCl_3$), δppm 0.55 (3H, s, 18-H_3) 0.78 (6H, dd, 26-H_3 and 27-H_3) 0.87 (3H, d, 21-H_3) 0.93 (3H, d, 28-H_3) 3.94 (1H, m, 3-H) 4.82 (1H, m (sharp), 19-H), 5.04 (1H, m (sharp), 19-H), 6.04 (1H, d, 7-H) 6.24 (1H, d, 6-H).

To a stirred solution of vitamin D_4 (IX) (3.0 g, 7.5 mmol) in 10 ml of dry pyridine was added freshly recrystallized p-

toluenesulfonyl chloride (3.6 g, 19 mmol) at 0°C. The reaction mixture was stirred at 5°C for 24 hours, and was then quenched by pouring the mixture over ice and saturated $NaHCO_3$ (100 ml) with stirring. The aqueous suspension was extracted with CH_2Cl_2 (3 x 300 ml). The combined organic extracts were washed with 10% HCl (3 x 200 ml), saturated $NaHCO_3$ (3 x 200 ml) and saturated NaCl (2 x 200 ml), dried over $MgSO_4$ and concentrated _in vacuo_ to yield 3.5 g. (84%) of the novel intermediate compound vitamin D_4 tosylate (X) and had the following characteristics: ^1H NMR (400 MHz, $CDCl_3$), δppm 0.54 (3H, _s_, 18-H_3) 0.78 (6H, _dd_, 26-H_3 and 27-H_3) 0.87 (3H, _d_, 21-H_3), 0.96 (3H, _d_, 28-H_3) 2.45 (3H, _s_, CH_3 (tosylate) 4.68 (3H, _m_, 3-H) 4.82 (1H, _m_ (sharp), 19-H) 5.04 (1H, _m_ (sharp), 19-H), 5.95 (1H, _d_ 7-H), 6.09 (1H, _d_, 6-H) 7.34 and 7.79 (4H, _d_, aromatic).

To a stirred suspension of $NaHCO_3$ (17.0 g, 202 mmol) in methanol (200 ml) a solution of vitamin D_4 tosylate (X) (3.5 g, 6.3 mmol) in dry CH_2Cl_2 (10 ml) was added dropwise. The reaction mixture was refluxed overnight under argon, and then cooled to room temperature and concentrated _in vacuo_ to about 50 ml. The reaction concentrate was diluted with ether (600 ml), washed with water (3 x 300 ml), dried over $MgSO_4$ and concentrated _in vacuo_. The residue was passed through a silica gel column and eluted with 10% ethyl acetate in hexane to afford the novel intermediate compound 3,5 cyclovitamin D_4 (XI) (heavy oil) with a yield of 1.5 g. (58%) and had the following characteristics: ^1H NMR (400 MHz, $CDCl_3$), δppm 0.56 (3H, _s_, 18-H_3) 0.78 (6H, _dd_, 26-H_3 and 27-H_3), 0.87 (3H, _d_, 21-H_3), 0.94 (3H, _d_, 28-H_3), 3.28 (3H, _s_, OCH_3) 4.2 (1H, _d_, 6-H), 4.91 (1H, _m_ (sharp), 19-H), 4.98 (1H, _d_ 7-H), 5.08 (1H, _m_ (sharp), 19-H).

Anhydrous tert-butyl hydroperoxide in toluene (3M) (2.6 ml, 7.8 mmol) was added to a stirred suspension of selenium dioxide (0.22 g, 2 mmol) in dry CH_2Cl_2 (150 ml) in a three necked flask. The mixture was stirred for three hours under argon. Pyridine (0.3 ml, 3.7 mmol) was then added, and cyclovitamin D_4 (XI) (1.5 g, 3.6 mmol) was then introduced as a solution in CH_2Cl_2 (50 ml). After stirring for thirty minutes, 10% aqueous NaOH solution (200 ml) was added. The reaction mixture was then diluted with ether (500 ml) and the phases were separated. The organic phase was washed with 10% NaOH (3 x 200 ml), water (2 x 200 ml) and

saturated NaCl solution (2 x 200 ml), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was absorbed on a silica gel column and eluted with 30% ethyl acetate in hexane to afford 0.45 g. (29%) of the novel intermediate compound 1α-hydroxy 3,5-cyclovitamin D_4 (XII) (oil) and had the following characteristics: ^{1}H NMR (400 MHz, $CDCl_3$), δppm 0.54 (3H, *s*, 18-H_3) 0.78 (6H, *dd*, 26-H_3 and 27-H_3), 0.86 (3H, *d*, 21-H_3) 0.95 (3H, *d*, 28-H_3) 3.26 (3H, *s*, OCH_3) 4.2 (1H, *d*, 6-H), 4.22 (1H, *m*, 1-H), 4.95 (1H, *d* 7-H), 5.18 (1H, *d*, 19-H) 5.25 (1H, *d*, 19-H).

A solution of 1α-hydroxy 3,5-cyclovitamin D_4 (XII) (0.45 g, 1.05 mmol) in a solution of dimethyl sulfoxide (4.5 ml) and glacial acetic acid (3.6 ml) was heated to 50°C under argon for one hour. The reaction mixture was then poured over ice and saturated $NaHCO_3$ solution (100 ml), and extracted with ether (3 x 200 ml). The combined ether extracts were washed with saturated $NaHCO_3$ solution (3 x 200ml), water (3 x 200 ml) and saturated NaCl solution (3 x 200 ml), dried over $MgSO_4$, concentrated *in vacuo* to give a mixture containing 5,6-cis and 5,6-trans 1α-hydroxy vitamin D_4 (about 4:1 by ^{1}H NMR) with a yield of 0.4g, (92%). The mixture of 5,6-cis and 5,6-trans 1α-hydroxy vitamin D_4 (0.4 g, 0.97 mmol) was dissolved in ethyl acetate (25 ml) and treated with freshly recrystallized maleic anhydride (0.08 g, 0.8 mmol). This reaction mixture was heated to 35°C under argon for 24 hours. After evaporation of the solvent *in vacuo*, the crude mixture was chromatographed over a silica gel column using ethyl acetate and hexane (1:1) as eluent, to afford the novel active form of vitamin D_4, 5,6-cis 1α-hydroxy vitamin D_4 (XIII) with a yield of 90 mg (23%) and had the following characteristics: m.p.: 128-130°C; IR ν_{max} (Neat): 3400 cm^{-1} (OH stretching); ^{1}H NMR (400 MHz, $CDCl_3$), δppm 0.55 (3H, *s*, 18-H) 0.79 (6H, *dd*, 26-H_3 and 27-H_3) 0.87 (3H, *d*, 21-H_3) 0.94 (3H, *d*, 28-H_3), 4.24 (1H, *m*, 3-H), 4.44 (1H, *m*, 1-H), 5.02 (1H, *m* (sharp), 19-H), 5.34 (1H, *m* (sharp), 19-H), 6.02 (1H, *d* 7-H), 6.4 (1H, *d*, 6-H); Mass spectrum [CI] m/e (relative intensity): 415 (M+1, 41%) 397, (M+1-OH 100%), 379 (27%), 135 (22%).

Example 2: Biological testing of 1α-hydroxy vitamin D_4

Male weanling rats (Holtzman strain, Holtzman Company, Madison, Wisconsin) were fed a vitamin D deficient diet

containing adequate calcium (0.47%) and phosphorus (0.3%). Within three to four weeks, this diet induces an extreme vitamin D deficiency characterized by low serum calcium and poor growth. After four weeks on this diet, the rats had serum calcium values less than 7 mg/dl. The rats were then separated into four groups and orally administered either 1α-hydroxy vitamin D_4 in a vehicle such as coconut oil or the vehicle (control) for each of 14 days. Twenty-four hours after the last dose, the rats were killed and the blood calcium measured by a standard laboratory technique. The results of these determinations are shown in Table 1.

TABLE 1

Increase in Serum Calcium Concentration

Compound	Dose (μg/kg/day)	Number of rats	Serum calcium concentration (mg/dl) ± Standard Deviation
Control	-	10	6.1±0.48
1α-OH-D_4	0.042	8	7.1±0.80
1α-OH-D_4	0.250	7	11.6±0.45
1α-OH-D_4	1.500	9	12.7±0.37

The data of Table 1 indicate that 1α-hydroxy vitamin D_4 is effective at increasing serum calcium in the vitamin D deficient rat and that the response appears to be dose dependent. Surprisingly, the level of the response appears to compare favorably to that reported by Wientroub, et. al., for 1,25 dihydroxy vitamin D_3 administered to vitamin D deficient rats under experimental conditions similar to those described above. See, Wientroub, S., Price, P.A., Reddi, A.H., "The Dichotomy in the Effects of 1,25 dihydroxy vitamin D_3 and 24,25 dihydroxy vitamin D_3 on Bone Gamma-Carboxyglutamic Acid-Containing Protein in Serum and Bone in vitamin D-Deficient Rats," Calcif. Tissue Int. (1987) 40:166-172.

Example 3: **Toxicity tests**

The acute oral toxicity of 1α-OH-D_4 in rats was assessed by determining the mean lethal dose (LD_{50}) using a well-known

method. Rats were fed a standard laboratory diet for 8-10 weeks. Five animals of each sex were administered one oral dose of 1α-OH-D_4. The animals were observed for 14 days, and the number of deaths noted. The LD_{50} value was determined to be about 1.0 mg/kg in males and 3.0 mg/kg in females.

For comparison, the LD_{50} value for 1α-hydroxy vitamin D_2 under the same conditions was found by applicant's to be 1.7 and 1.8 mg/kg. in male and female rats, respectively. The toxicity of 1α-hydroxy vitamin D_2 has previously been reported as less than 1α-hydroxy vitamin D_3. Sjoden, G., Smith, C., Lindgren, U., and DeLuca, H.F., Proc. Soc. Experimental Biol. Med., 178:432-436 (1985).

Example 4: Generation and Isolation of 1,25-dihydroxy vitamin D_4

The 1α-hydroxy vitamin D_4 of the present invention is incubated with cultured human liver cells which metabolize the compound to several products including the metabolite 1,25 dihydroxy vitamin D_4. The 1,25 metabolite is isolated and purified by high pressure liquid chromatography and identified by gas-chromatography-mass spectrometry. Binding studies demonstrate that the 1,25 dihydroxy vitamin D_4 has good binding affinity for the mammalian vitamin D receptor protein indicating it is biologically active. The procedures used are similar to that described by Strugnell, et. al., Biochem. Pharm. Vol. 40:333-341 (1990).

Example 5: Generation and isolation of 1,24-dihydroxy vitamin D_4

Generation and isolation of 1,24 dihydroxy vitamin D_4 is accomplished as described in Example 4, above. The 1α-hydroxy vitamin D_4 of the present invention is incubated with cultured human liver cells which metabolize the compound to several products including the metabolite 1,24 dihydroxy vitamin D_4. The 1,24 metabolite is isolated and purified using high pressure liquid chromatography and identified by gas-chromatography-mass spectrometry. Binding studies with the new metabolite demonstrate that the metabolite has good binding affinity for the mammalian vitamin D receptor protein which indicates the drug is biologically active.

calcemia testing

are fed a commercial diet containing 0.8% d phosphorus (0.6%). The rats are divided into each group is orally administered daily either hicie such as coconut oil or the vehicie for 13 weeks. Twenty-four hours after the last are killed and their serum calcium determined by nod.

edure demonstrates that the serum calcium is unaffected or only slightly elevated at doses o 2.5 μg/kg/day.

Further biological testing

eanling rats are fed a diet deficient in vitamin D w calcium (0.02%). After a period of four weeks has e rats are divided into four groups and intravenously ed either 1α-OH D_4 in a vehicle such as ethanol or the control) alone. Sixteen hours after administration, are killed and the intestinal calcium transport by using everted duodenal sacs, following the method of d DeLuca, Am. J. Physiol. 216:1352-1359. lowing this procedure demonstrates stimulation of al calcium transport in a dose dependent manner.

e 8:

clinical study is conducted with postmenopausal porotic outpatients having ages between 55 and 75 years. tudy involves up to 120 patients randomly divided into e treatment groups, and continues for 12 to 24 months. Two he treatment groups receive constant dosages of 1α-vitamin D_4 .d.; two different dose levels above 3.0 μg/day) and the er group receives a matching placebo. All patients maintain normal intake of dietary calcium (500 to 800 mg/day) and frain from using calcium supplements. Efficacy is evaluated pre- and post-treatment comparisons of the patient groups ith regard to (a) total body, radial, femoral and/or spinal one mineral density as determined by x-ray absorptiometry (DEXA), (b) bone biopsies of the iliac crest, and (c) determinations of serum osteocalcin. Safety is evaluated by

comparisons of urinary hydroxyproline excretion, serum and urine calcium levels, creatinine clearance, blood urea nitrogen, and other routine determinations.

This study demonstrates that patients treated with 1α-vitamin D_4 exhibit significantly higher total body, radial, femoral and/or spinal bone densities relative to patients treated with placebo. The treated patients also exhibit significant elevations in serum osteocalcin. Bone biopsies from the treated patients show that 1α-vitamin D_4 stimulates normal bone formation. The monitored safety parameters confirm an insignificant incidence of hypercalcemia or hypercalciuria, or any other metabolic disturbance with 1α-vitamin D_4 therapy.

Example 9:

A clinical study is conducted with healthy postmenopausal women having ages between 55 and 60 years. The study involves up to 80 patients randomly divided into two treatment groups, and continues for 12 to 24 months. One treatment group receives a constant dosage of 1α-vitamin D_4 (u.i.d.; a dose level above 3.0 μg/day) and the other receives a matching placebo. The study is conducted as indicated in Example 2 above.

This study demonstrates that patients treated with 1α-vitamin D_4 exhibit reduced losses in total body, radial, femoral and/or spinal bone densities relative to baseline values. In contrast, patients treated with placebo show significant losses in these parameters relative to baseline values. The monitored safety parameters confirm the safety of long-term 1α-vitamin D_4 administration at this dose level.

Example 10:

A twelve-month double-blind placebo-controlled clinical trial is conducted with thirty men and/or women with renal disease who are undergoing chronic hemodialysis. All patients enter an eight-week control period during which time they receive a maintenance dose of vitamin D_3 (400 IU/day). After this control period, the patients are randomized into two treatment groups: one group receives a constant dosage of 1α-vitamin D_4 (u.i.d.; a dosage greater than 3.0 μg/day) and the other group receives a matching placebo. Both treatment groups

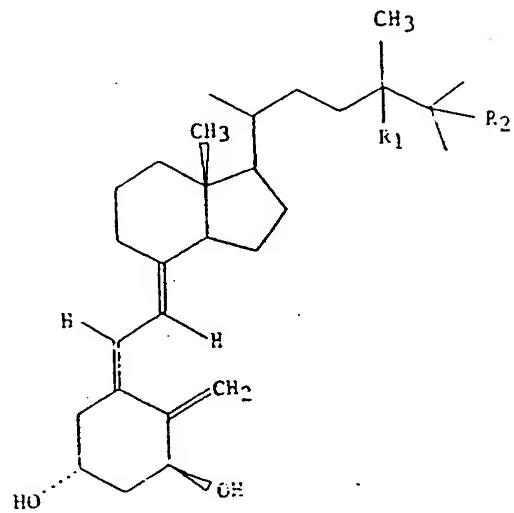
receive a maintenance dosage of vitamin D_3, maintain a normal intake of dietary calcium, and regrain from using calcium supplements. Efficacy is evaluated by pre- and post-treatment comparisons of the two patient groups with regard to (a) direct measurements of intestinal calcium absorption, (b) total body, radial, femoral and/or spinal bone mineral density, and (c) determinations of serum calcium and osteocalcin. Safety is evaluated by regular monitoring of serum calcium.

Analysis of the clinical data shows that 1α-vitamin D_4 significantly increases serum osteocalcin levels and intestinal calcium absorption, as determined by measurements using a single or double-isotope technique. Patients treated with this compound show normalized serum calcium levels, stable values for total body, radial, femoral and/or spinal bone densities relative to baseline values. In contract, patients treated with placebo show frequent hypocalcemia, significant reductions in total body, radial, femoral and/or spinal bone density. An insignificant incidence of hypercalcemia is observed in the treated group.

While the present invention has now been described and exemplified with some specificity, those skilled in the art will appreciate the various modifications, including variations, additions, and omissions, that may be made in what has been described. Accordingly, it is intended that these modifications also be encompassed by the present invention and that the scope of the present invention be limited solely by the broadest interpretation that lawfully can be accorded the appended claims.

CLAIMS:

1. The compound of the formula (I):



(I)

wherein R_1 is either H or OH and R_2 is either H or OH and salts, hydrates and solvates thereof.

2. The compound of claim 1, wherein said compound is 1α-hydroxy vitamin D_4.

3. The compound of claim 1, wherein said compound is 1,24 dihydroxy vitamin D_4.

4. The compound of claim 1, wherein said compound is 1,25 dihydroxy vitamin D_4.

5. The compound of claim 1, wherein said compound is biologically active.

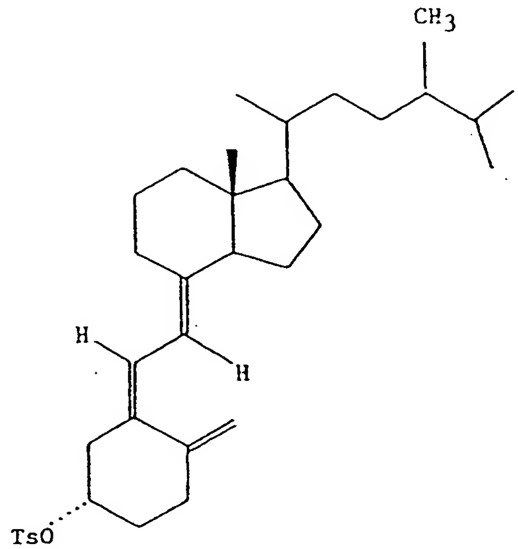
6. The compound of formula (I) according to claim 1, wherein R_1 is H or OH and R_2 is H or OH and wherein said compound exhibits biological activity approaching that of 1,25 vitamin D_3 and wherein said compound is less toxic than 1α-hydroxy vitamin D_3 as determined by comparative LD_{50} values in rats.

7. The compound of claim 6, wherein said compound is 1α-hydroxy vitamin D_4.

8. The compound of claim 6, wherein said compound is 1,25 dihydroxy vitamin D_4.

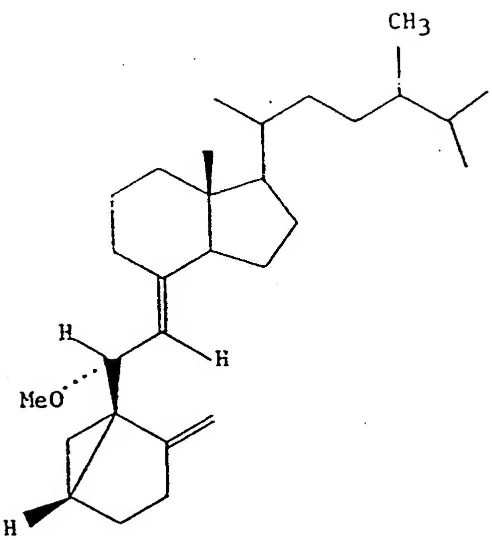
9. The compound of claim 6, wherein said compound is 1,24 dihydroxy vitamin D_4.

10. The vitamin D_4 tosylate compound of the formula (X):



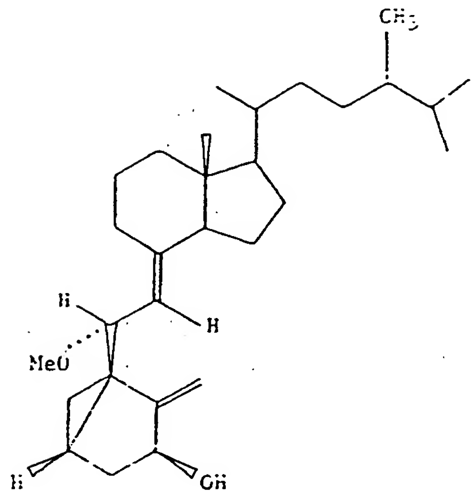
(X)

11. The 3,5 cyclovitamin D_4 compound of the formula (XI):



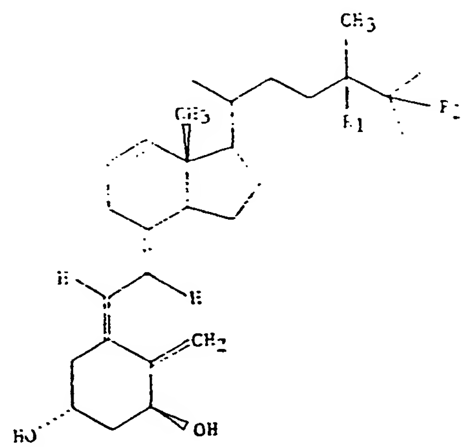
(XI)

12. The 1α-hydroxy 3,5 cyclovitamin D_4 of the formula (XII):



(XII)

13 A pharmaceutical composition, comprising an amount effective to increase serum calcium in a patient suffering vitamin D deficiency of a compound of the formula (I):



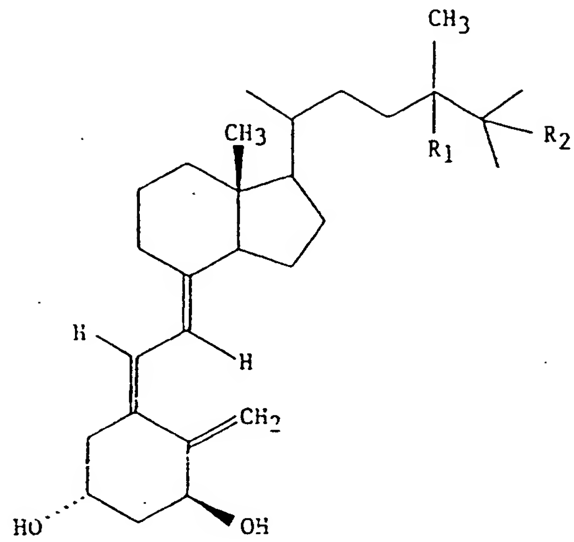
(I)

wherein R1 is either H or OH and R2 is either H or OH in combination with a pharmaceutically acceptable vehicle.

14 The pharamaceutical composition of claim 13, wherein said amount is administered orally.

15. A method of treating vitamin D deficiency induced diseases comprising administering to a patient suffering

therefrom an amount effective to treat the deficiency of a compound of the formula (I):



(I)

wherein R1 is either H or OH and R2 is either H or OH.

16. A method of preparing 1α-hydroxy vitamin D_4, comprising:

(a) tosylating vitamin D_4 to form vitamin D_4 tosylate;

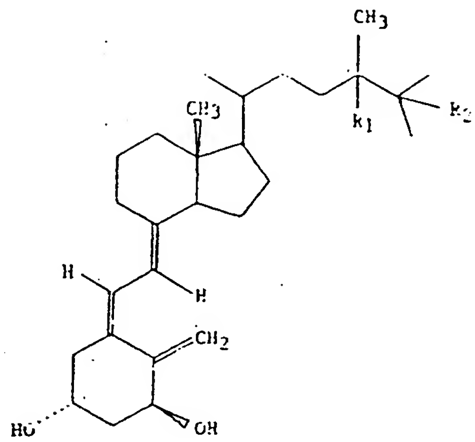
(b) solvolyzing the vitamin D_4 tosylate to form 3,5 cyclovitamin D_4;

(c) oxidizing the 3,5 cyclovitamin D_4 to form 1α-hydroxy 3,5 cyclovitamin D_4; and

(d) sequentially solvolyzing and subjecting to a Diels-Alder reaction the 1α-hydroxy-3,5 cyclovitamin D_4 to form 1α-hydroxy vitamin D_4.

17. A method for treating hypocalcemia in a mammal, comprising administering to a mammal an amount, effective to

increase serum calcium in the mammal, of a compound having the formula (I):



(I)

wherein R1 is either H or OH and R2 is either H or OH.

18. The method of claim 17, wherein said mammal suffers a vitamin D deficiency.

19. The method of claim 17, wherein said compound is administered in a daily dose of about 0.04 μg to about 1.5 μg per kg of body weight of the treated mammal.

20. The method of claim 17, wherein the hypocalcemia is vitamin D dependent rickets, hypoparathyroidism, post-operative renal osteodystrophy, liver cirrhosis, or steatorrhoea.

21. A method of producing vitamin D_4 tosylate, comprising reacting vitamin D_4 with toluenesulfonyl chloride in the presence of dry pryridine.

22. A method of producing 3,5 cyclovitamin D_4, comprising subjecting vitamin D_4 tosylate to buffered solvolysis.

23. A method of producing 1α-hydroxy 3,5 cyclovitamin D_4, comprising allylically oxidizing the 3,5 cyclovitamin D_4 with selenium dioxide.

24. A method of producing 1α-hydroxy vitamin D_4, comprising solvolizing the 1α-hydroxy 3,5 cyclovitamin D_4 with a mixture of dimethylsulfoxide and an organic acid to form an admixture of the 5,6 cis 1α-hydroxy and 5,6 trans 1α-hydroxy vitamin D_4 and subjecting the admixture to a Diels-Alder reaction forming an adduct of the 5,6 trans 1α-hydroxy vitamin D_4 to yield the 1α-hydroxy vitamin D_4.

25. A method of producing 1α-hydroxy vitamin D_4,

comprising: reducing ergosterol to 22,23 dihydroxyergosterol, irradiating the 22,23 dihydroxyergosterol to form vitamin D_4, and hydroxylating vitamin D_4 to form 1α-hydroxy vitamin D_4.

26. A method of producing 1α-hydroxy vitamin D_4, comprising:

(a) acetylating ergosterol to form ergosteryl acetate;

(b) hydroxyhalogenating the ergosteryl acetate to form 3β-acetoxy-6α-chloroergosta-7,22-dien-5α-ol;

(c) reducing and reacylating the 3β-acetoxy-6α-ergosta-7,22-dien-5α-ol to 3β-acetoxyergosta-7,22-dien-5α-ol;

(d) hydrogenating the 3β-acetoxyergosta-7,22-dien-5α-ol to form 3β-acetoxyergsto-7-en-5α-ol;

(e) reducing the 3β-acetoxyergsto-7-en-5α-ol to form 22,23 dihydroergosteryl acetate;

(f) reducing the 22,23 dihydroergosteryl acetate to 22,23 dihydroergosterol;

(g) irradiating 22,23 dihydroergosterol to form vitamin D_4;

(h) tosylating vitamin D_4 in the presence of dry pryridine to form vitamin D_4 tosylate;

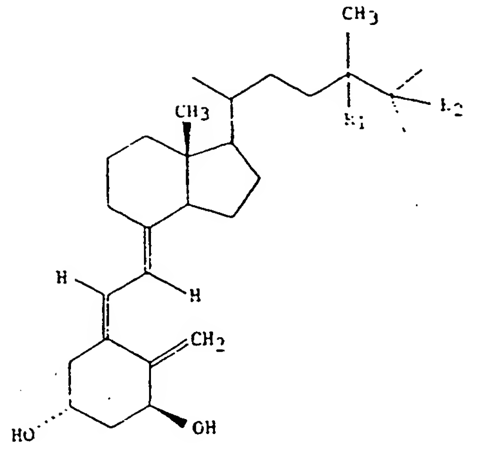
(i) solvolyzing vitamin D_4 tosylate to form 3,5 cyclovitamin D_4;

(j) allylically oxidizing the 3,5 cyclovitamin D_4 with selenium dioxide to form 1α-hydroxy vitamin D_4; and

(k) solvolyzing the 1α-hydroxy 3,5 cyclovitamin D_4 with a mixture of dimethylsulfoxide and an organic acid to form an admixture of the 5,6 cis 1α-hydroxy and 5,6 trans 1α-hydroxy vitamin D_4 and forming a Diels-Alder adduct of the 5,6 trans 1α-hydroxy vitamin D_4 to yield 1α-hydroxy vitamin D_4.

27. A pharmaceutical composition for controlling calcium metabolism comprising a physiologically acceptable vehicle and

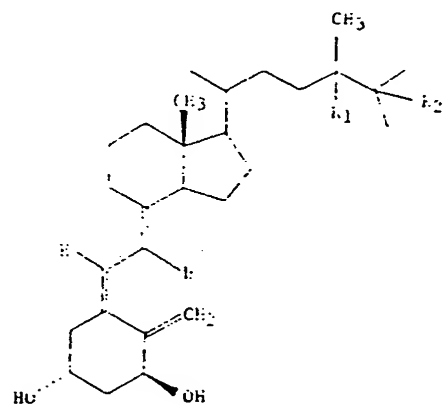
an effective amount of at least one compound of formula (I):



(I)

wherein R1 is either H or OH and R2 is either H or OH.

28. A prophylactic or therapeutic pharmaceutical composition for vitamin D deficient diseases, comprising a physiologically acceptable vehicle and an effective amount of at least one compound of formula (I):

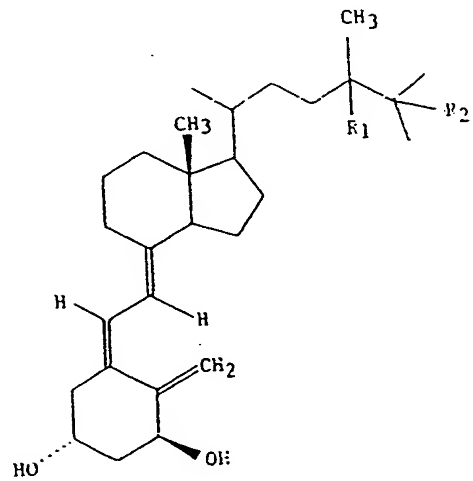


(I)

wherein R1 is either H or OH and R2 is either H or OH.

29. A method of controlling calcium metabolism in a mammal, comprising administering to a mammal a pharmaceutically

effective amount of a compound of formula (I):



(I)

wherein R1 is either H or OH and R2 is either H or OH.

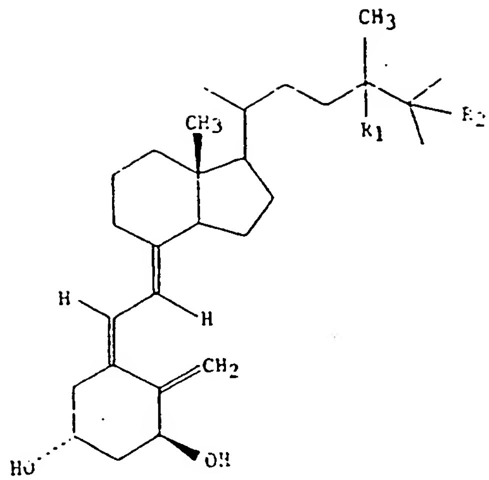
30. The method of claim 30, wherein said administering step is done orally, intramuscularly or intravenously.

31. The method of claim 30, wherein the effective amount is about 0.04 μg to about 1.5 μg per kg of body weight of the treated mammal.

32. A feed for mammals comprising at least one compound of the formula (I) wherein R1 is either H or OH and R2 is either H or OH wherein normal consumption of the feed by the mammals provides about 0.01 to about 0.5 μg/kg/day of said compound.

33. A pharmaceutical composition, comprising, an amount, effective to treat abnormal calcium metabolism in a mammal suffering from vitamin D deficiency, of a compound of the

formula (I):



(I)

wherein R_1 is either H or OH and R_2 is either H or OH in combination with a pharmaceutically acceptable vehicle.

34. A method for treating vitamin D deficiency-induced hypocalcemia, comprising:

(a) reducing ergosterol, under such conditions and in sufficient quantity to produce 22,23 dihydroergosterol;

(b) irradiating the 22,23 dihydroergosterol to produce vitamin D_4;

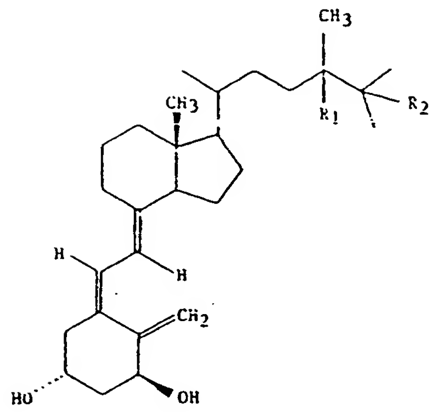
(c) hydroxylating the vitamin D_4 under such conditions and in sufficient quantity to produce 1α-hydroxy vitamin D_4;

(d) purifying the vitamin D_4; and

(e) administering to a mammal suffering from vitamin D deficiency-induced hypocalcemia an amount effective to increase serum calcium of 1α-hydroxy vitamin D_4 in admixture with a pharmaceutically acceptable vehicle.

35. A pharmaceutical composition for treating osteoporosis comprising a physiologically acceptable vehicle and an effective

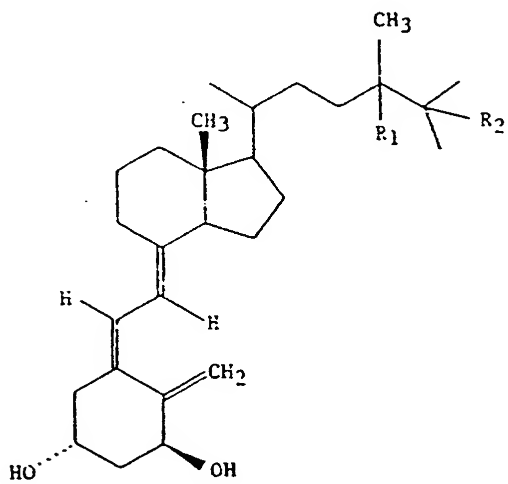
amount of a compound of the formula (I):



(I)

wherein R1 is either H or OH and R2 is either H or OH.

36. A method of treating osteoporosis, comprising administering to a patient suffering therefrom an amount effective to treat the osteoporosis of a compound of the formula (I):



(I)

wherein R1 is either H or OH and R2 is either H or OH.

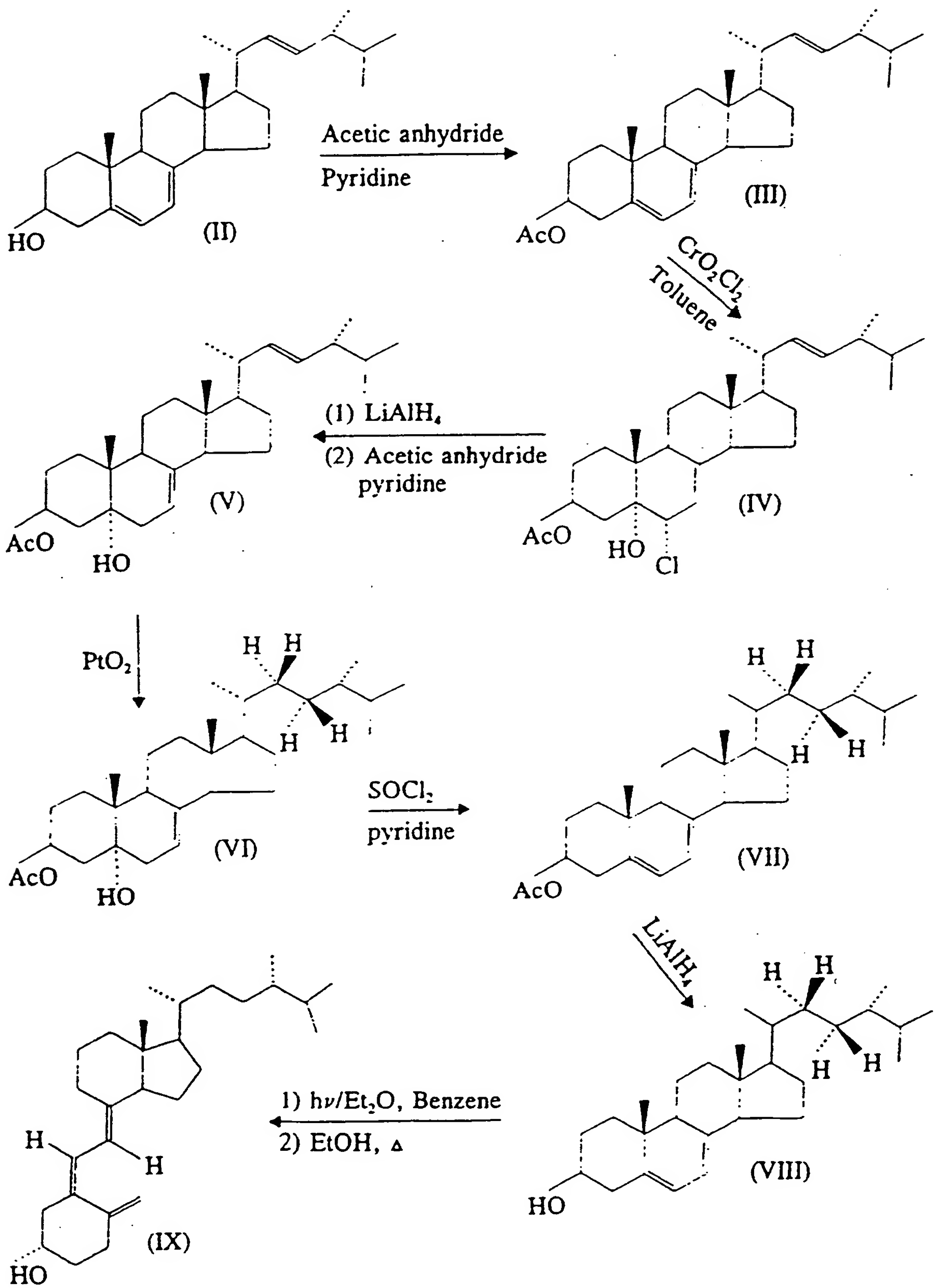

Acetic anhydride
Pyridine
HO
(II)
AcO
(III)
CrO_2Cl_2
Toluene
(1) $LiAlH_4$
(2) Acetic anhydride
pyridine
AcO
HO
(V)
AcO
HO
Cl
(IV)
PtO_2
AcO
HO
(VI)
$SOCl_2$
pyridine
AcO
(VII)
$LiAlH_4$
1) hν/Et_2O, Benzene
2) EtOH, Δ
HO
(IX)
HO
(VIII)

FIGURE 1

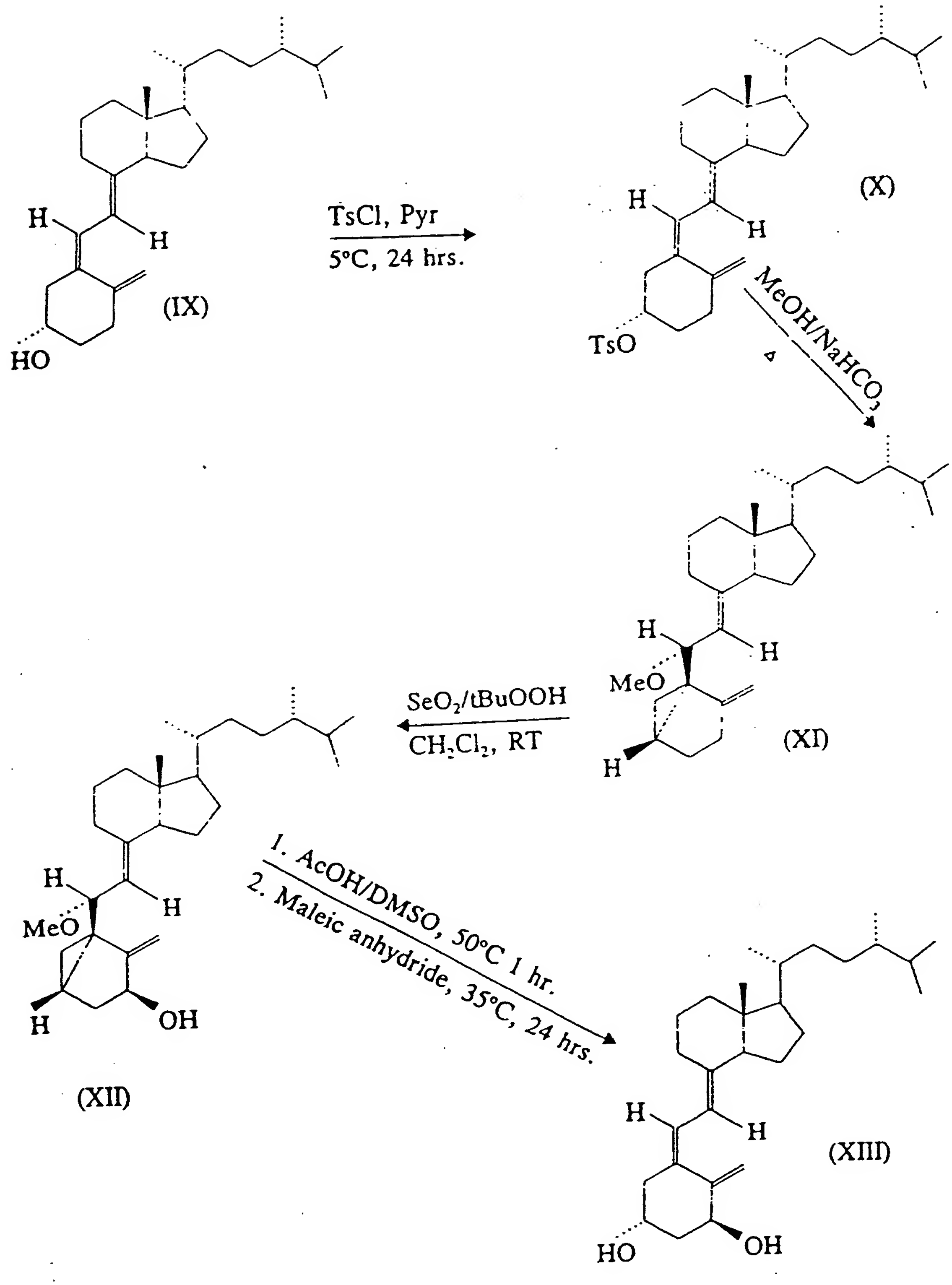

TsCl, Pyr
5°C, 24 hrs.
(IX)
(X)
MeOH/NaHCO$_3$
Δ
(XI)
SeO$_2$/tBuOOH
CH$_2$Cl$_2$, RT
(XII)
1. AcOH/DMSO, 50°C 1 hr.
2. Maleic anhydride, 35°C, 24 hrs.
(XIII)
HO
TsO
MeO
OH
H

FIGURE 2

International Application No PCT/US91/06865

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C07C 9/00, A61K 31/59

US CL 552/653, 514/168

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	552/653, 514/168

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [*]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US, A, 4,202,859 (DULUCA ET AL.) 13 MAY 1980	1-9,13-15, 17-20,27-31, 33-36
Y	DELUCA ET AL. Arch. Biochem. and biophys. 124, 122-128 (1968) Synthesis, Biological Activity and Metabolism of 22,23 ^{3}H Vitamin D_4.	1-9,13-15,17-20,27-31,33-36
Y	Windavs, et al. 2. Physiol. Chem. 247, 1937, pp. 185 to 188. Uber das Krystallisierte Vitamin D_4.	1-9,13-15,17-20,27-31,33-36

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
25 OCTOBER 1991	20 DEC 1991
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Mukund J. Shah

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE[1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ______ because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers ______ because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers ______, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING[2]

This International Searching Authority found multiple inventions in this international application as follows:

Group I, Claims 1-9,13-15,17-20, 27-31 and 33-36 vitamin D_4.
Group II, claims 11 and 21, tosylated vitamin D_4.
Group III, 3,5 Cyclovitamin D_4, claims 12-22.
Group IV, Claims 16 and 24, first method of preparing vitamin D_4.

See extra sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

1-9,13-15,17-20,27-31, and 33-36

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remarks on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.